Innova Biosciences
making science easier

InnovaCoat® GOLD Hydrazide (10 OD) 40nm Conjugation Kit

Applicable to:

280-0100 1 vial (to label 10-30ug Ab)
280-0000 3 vials (to label 10-30ug Ab each)
280-0300 1 vial (to label 30-90ug Ab)

Release 4 27/01/2015

Introduction

Antibodies can be covalently attached quickly and easily, in a *site-specific* fashion, to ultra stable InnovaCoat* GOLD nanoparticles functionalized with hydrazides (1 min hands-on time and 30min incubation).

While antibodies in their native state do not contain aldehyde groups, it is easy to introduce aldehydes by mild oxidation of the sugar chains located in the Fc domain. Covalent attachment of aldehyde-modified antibodies to hydrazide surfaces therefore attaches the antibody via the Fc region, and orients the antigen-binding domains in such a way as to maximize antigen binding, which can lead to enhanced assay performance.

This kit provides all of the materials required to conjugate three antibodies in site specific manner to InnovaCoat GOLD. Sufficient material is provided for small scale trial conjugations and then scale up of the best reagent.

**InnovaCoat is a special surface coating material for gold nanoparticles which remains firmly anchored even under the most extreme conditions. InnovaCoat GOLD derivatives display unrivalled colloidal stability.*

Kit contents

Contents of the site-specific labeling kit:

1 or 3 amber glass vials of Antibody Oxidation Reagent

1 vial (1.5ml) or bottle (4ml) of 10.0 OD InnovaCoat GOLD-Hydrazide

1 Vial of Antibody diluent (500µl)

1 Bottle of Wash/Coupling Buffer (20ml or 50ml)

3 or 1 separating columns

Shipping conditions

The kit is shipped at ambient temperature.

Store the following at -20°C:
Antibody Oxidation Reagent

Store the following at +4°C:
InnovaCoat GOLD-Hydrazide

Antibody diluent

Wash/Coupling Buffer

Store the following at room temperature:
Separating columns

Considerations prior to activation step

The primary consideration is whether the antibody is in a suitable buffer to allow aldehyde groups to be introduced into the Fc domain. Please see the table below for buffer recommendations.

Buffer components	
pH	7.0
100mM phosphate	✓
Non-buffering salts *(e.g. sodium chloride)*	✓
Chelating agents *(e.g.EDTA)*	✓
Sugars (sucrose, glucose)	×
Glycerol	×
Thiomersal	×
Thimerosal	×
Merthiolate	×
Sodium Azide[1]	×
BSA[1,2]	×
Gelatin[1,2]	×
Tris	×
Glycine	×
Nucleophilic components *(Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)*	×

Activation of the antibody

(i) Adjust (if required) the concentration of the antibody in phosphate buffer to 1mg/ml using the antibody diluent provided in the kit.

(ii) Add

- 100µl (1 and 3 reaction small scale kits)
- 300µl (1 reaction larger scale kit)

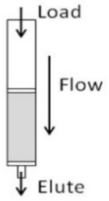
of the antibody into the vial of Oxidation Reagent. Mix gently and incubate for 30 min in the dark at room temperature.

(iii) Proceed to desalting procedure.

Desalting procedure

Use one column per antibody. The columns are designed for single use. Discard after use.

(i) Secure a separating column in a vertical position. Remove the two caps (remove the upper one first) and allow the storage liquid to flow through the column to waste.



(ii) Equilibrate the column with the Wash/Coupling Buffer by filling the column up to the very top and allowing the liquid to flow through under gravity. Discard the flow-through. Repeat a further 4 times. When the last portion of buffer has passed into the column, move to the next step.

(iii) After the oxidation reaction has completed add all of the activated antibody to the top of the column and allow the liquid (i.e. 100µl or 300µl) to pass completely into the column.

(iv) Add

- 600µl (1 and 3 reaction small scale kits)
- 400µl (1 reaction larger scale kit)

of Wash/Coupling Buffer to the top of each column. This liquid is required to push the activated material to the base of the column. Allow this liquid to pass completely into the column before proceeding to the next step.

(v) Place a 0.5ml or 1.5ml collection tube (not supplied) under the column. Add

- 200µl (1 and 3 reaction small scale kits)
- 450µl (1 reaction larger scale kit)

of Wash/Coupling Buffer to the top of the column.

(vi) Collect the eluate from the column into the collection tube. This tube now contains the activated antibody free of excess Oxidation Reagent and other chemicals.

The antibody concentration in the eluate will be approximately 0.5mg/ml (small scale kits), and 0.65mg/ml (larger scale kit).

Storage of activated antibody

The activated antibody should be used within 2 hours. For longer term storage (weeks) divide the activated antibody in aliquots and store at -80°C to avoid multiple freeze-thaw cycles.

Conjugation to InnovaCoat GOLD hydrazide

The kit may be used in different ways to suit the particular aims of your experiment. The antibody can be tested at different antibody-gold ratios and/or at different scales of reaction.

The optimum amount of antibody (which will influence the number of molecules per particle) may be application-dependent and must be determined by experimentation. Around 10-30 ug of aldehyde-activated IgG antibody per ml of 10 OD InnovaCoat GOLD-Hydrazide is likely to give optimal results.

The procedure below is for conjugation of 10ug of antibody to 1ml InnovaCoat GOLD hydrazide, and can be varied simply be adjusting the amount of antibody added at step ii).

i) Remove 1ml of InnovaCoat GOLD-Hydrazide from the stock bottle and allow to warm to room temperature.

ii) Add 10ug of the activated antibody (20ul of antibody at 0.5mg/ml, 15ul of antibody at 0.65mg/ml). Leave the mixture standing for 30 minutes at room temperature (20-25°C); longer incubation time has no negative effect on the conjugate.

iii) After the conjugation, if you wish to exchange the InnovaCoat GOLD conjugate into a specific buffer for your assay or test, centrifuge the conjugate in a microfuge at 9,000g for 6 minutes. Carefully remove the supernatant and add your preferred buffer. The buffer should not contain thiols; all other common lab materials are acceptable after the conjugate has formed.

For additional applications please contact our Technical Support: technical.enquiries@innovabiosciences.com

Measuring antibody concentration

We strongly recommend that protein values should be determined using an absorbance at 280nm.

For an IgG using a 1cm light path an OD280 of 1.0 is equivalent to an antibody concentration of 0.714mg/ml.

When using Bradford-type protein assay reagents it is important to use an IgG standard curve. The absorbance generated by this type of reagent is dependent on the protein used. For example, using a BSA standard curve to determine the protein concentration of an IgG solution will result in a 2.3-fold under-estimate of the IgG concentration.

Related products/services

InnovaCoat® GOLD-Maleimide

3 Reaction 40nm Mini Kit (270-0005)

1 Reaction 40nm Midi Kit (270-0015)

The InnovaCoat® GOLD-Maleimide conjugation kit allows thiolated molecules (e.g. antibodies, Fab' fragments, or other sulfhydryl-containing biomolecules) to be covalently attached to ultra-stable InnovaCoat® GOLD nanoparticles with just simple pipetting operations.

Abure Antibody Purification kits

Commercially available antibodies often contain substances (e.g. BSA, glycine, tris, and azide) that interfere in labeling reactions. Innova Biosciences has developed a range of purification kits which allow for easy and rapid purification of antibodies from any buffer formulation. All products are easy to use and are fully compatible with our nanoparticle kits.

InnovaCoat® GOLD Custom Services

Including optimization of covalent antibody conjugates, covalent attachment of small molecules and ligands.